

The three-dimensional similarity between a dimeric antiparallel extended structure and a β -turn folded form of enkephalin

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The three-dimensional similarity between two fundamental conformations, a dimeric antiparallel extended structure and a type I' β -turn folded form, of enkephalin was examined by computer graphic and empirical energy calculation methods. By the rotation of Tyr and Phe side chains, one half of the former structure could mimic the three-dimensional form of the latter without a large loss of conformational energy. This result provides a new idea for considering the conformation of enkephalin suitable for the multiple opioid receptors. The active conformation of enkephalin for μ - and δ -opioid receptors is discussed in the light of the present study.

Enkephalin; β -Turn conformation; Extended dimeric conformation; Energy calculation; Conformational similarity; Selectivity

1. INTRODUCTION

Pharmacological and biochemical studies of opioid peptides have revealed the existence of several subclasses of opioid receptors [1–4]. Among them, the μ - and δ -opioid receptors have been most widely discussed. Despite numerous efforts, however, the structural characteristics of opioids suitable for the respective receptors remain to be elucidated.

Recently, the crystal structures of two endogenous opioid pentapeptides, Met⁵- and Leu⁵-enkephalins, have been analyzed by X-ray diffraction methods [5–12], and two different forms have been characterized as the fundamental conformations of enkephalin: a type I' β -turn folded form and a dimeric antiparallel extended structure (see fig.1). These two conformations

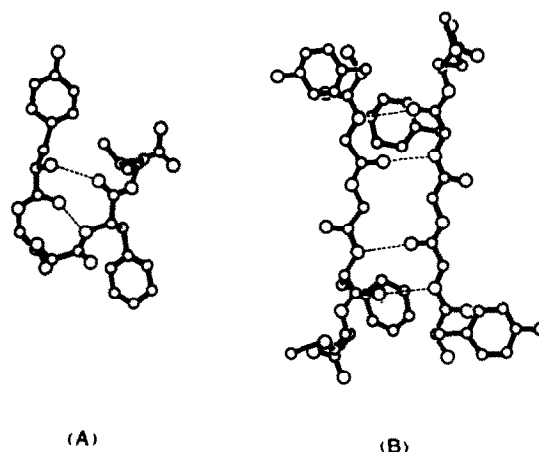


Fig.1. Two fundamental conformations of enkephalin. (A) Type I' β -turn folded form which has two intramolecular hydrogen bonds (from [7]); (B) a dimeric antiparallel extended structure which is stabilized by four intermolecular hydrogen bonds (from [12]). Structures A and B are denoted as forms I and II in the text, respectively.

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(henceforth denoted as forms I and II, respectively) have also been suggested to be predominant in a solution with a conformational equilibrium [13].

Although it is well known that enkephalin binds to both μ - and δ -receptors [14], it is at present unclear whether the same or a different conformation of enkephalin binds to these receptors. The present report suggests the possibility that either form I or II can simultaneously bind to both receptors.

2. METHODS AND PROCEDURES

Atomic coordinates obtained by X-ray crystal analyses were used for this study: form I was built from Leu⁵-enkephalin [7] and form II from Met⁵-enkephalin [12]. As is obvious from fig.1, the width between the antiparallel-running backbone chain of form I is almost the same as that of form II (4.5 Å). To inspect the three-dimensional similarity between both forms, therefore, the relative dispositions of Tyr¹, Phe⁴ and Met⁵ or Leu⁵ side chains were considered. The similarity was monitored by the three distances O(Tyr¹)-C_α(Gly³), O(Tyr¹)-C_γ(Phe⁴) and C_α(Gly³)-C_γ(Phe⁴), where O(Tyr¹), C_α(Gly³) and C_γ(Phe⁴) denote the phenolic oxygen atoms of Tyr¹, C_α atom of Gly³ and C_γ atom of Phe⁴, respectively. These amino acid residues are absolutely necessary for the activity emergence of enkephalin [15].

We attempted to fit the conformation of form II to form I by rotation of the Tyr¹ and Phe⁴ side chains. Both conformations were then superimposed by the shift of form I so as to minimize the value of $(\sum_{i=1}^N di)/N$, where di is the distance between the C_α atoms of the i -th residues in both forms, and N is the number of residues (= 5).

The energy change accompanying the rotation was calculated by the empirical PPF (partitioned potential function) method. The energy functions involved in the calculations were nonbonded, electrostatic and torsional energies, respectively. Details of the calculation procedure and the parameters used for the calculations have been presented in [16].

3. RESULTS AND DISCUSSION

In fig.2 is shown the energy variation accompa-

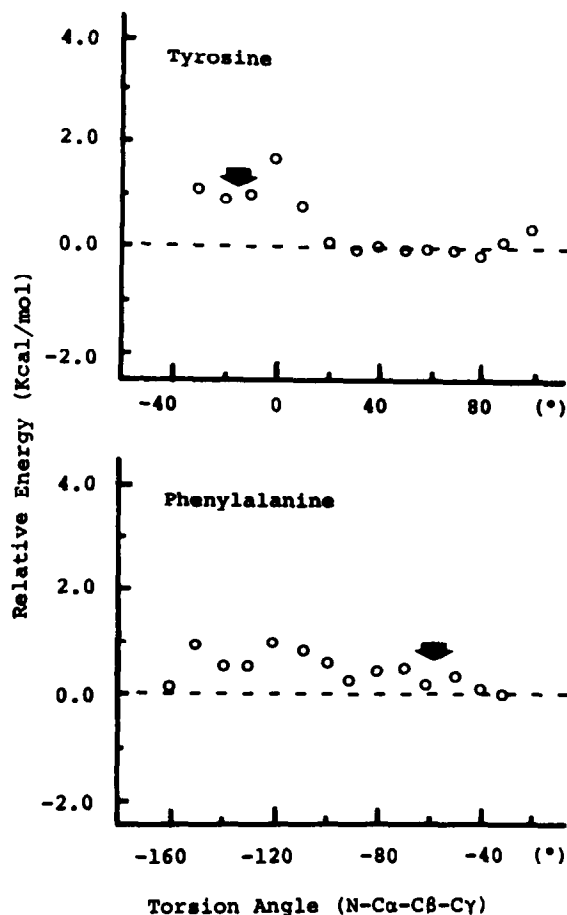


Fig.2. Relative energy of profiles of form II accompanying rotation of angle χ^1 of Tyr or Phe side chains. The profile is represented as the energy difference from the energy at $\chi^1 = 60^\circ$ for Tyr, or $\chi^1 = -40^\circ$ for Phe, respectively. The arrow indicates the torsion angle used for the superimposition.

nying the rotation (χ^1) about the C_α-C_β bond of the Tyr¹ or Phe⁴ side chain in form II; the conformation about the C_β-C_γ bond was optimized only within $\pm 4^\circ$ of the value observed in the crystal structure, because of the values being nearly equal (-89 and 95° for Tyr¹ and Phe⁴ of form I and -89 and -87° for those of form II, respectively) and being in an energetically stable region [17,18]. Fig.2 shows that the rotation about the C_α-C_β bond does not significantly make the extended dimer structure unstable. A maximum energy gap of 1.81 kcal/mol would be compensated by the proper external factors such as the interaction with

opioid receptors ('Zipper' models for ligand binding [19]). The high flexibilities of Tyr¹ and Phe⁴ side chains, along with those of Met⁵ or Leu⁵, have also been supposed from the crystal structures; these side chains are in the various conformation states.

Fig.3 shows the three-dimensional similarity between forms I and II, where the former was superimposed on the latter. One half of form II is nicely fitted to form I of enkephalin. The distances O(Tyr¹)–C_α(Gly³), O(Tyr¹)–C_γ(Phe⁴) and C_α(Gly³)–C_γ(Phe⁴) are 11.28, 11.75 and 4.60 Å for form I and 11.80, 10.93 and 5.04 Å for form II, respectively, and $(\sum_{i=1}^N d_i)/N$ is 1.20 Å. Although the relative orientation of each Tyr¹ aromatic ring with respect to its backbone chain is different, the positions of phenolic oxygen atoms are almost in the same site (the deviation between their oxygen atoms is 1.30 Å).

The opioid selectivity for the μ -receptor has been well discussed, based on the conformational

similarity between form I of enkephalin and μ -specific morphine [5,7,13]. On the other hand, the conformation of enkephalin suitable for δ -opioid receptors could be supposed to be form II, as judged from the various data; for example, the dimerization of μ -specific des-Leu⁵-[D-Ala²,Phe-NH₂]²enkephalin is converted into the δ -specific form [20,21], and the stable conformation of δ -specific deltaxenkephalin is predominantly in an extended structure (Ishida, T. et al., in preparation). Provided that forms I and II of enkephalin are the active conformations for μ - and δ -receptors, respectively, the present results imply that form II can also interact with the μ -receptor, and alternately, two enkephalins of form I, which are nearly related by a diad symmetry, with the δ -receptor; in other words, both forms of enkephalin can bind simultaneously to μ - and δ -receptors. This hypothesis can account well for the fact that enkephalin analogues with pure μ or δ activity have not yet been synthesized and, further, why intact enkephalin shows activity with μ - and δ -receptors.

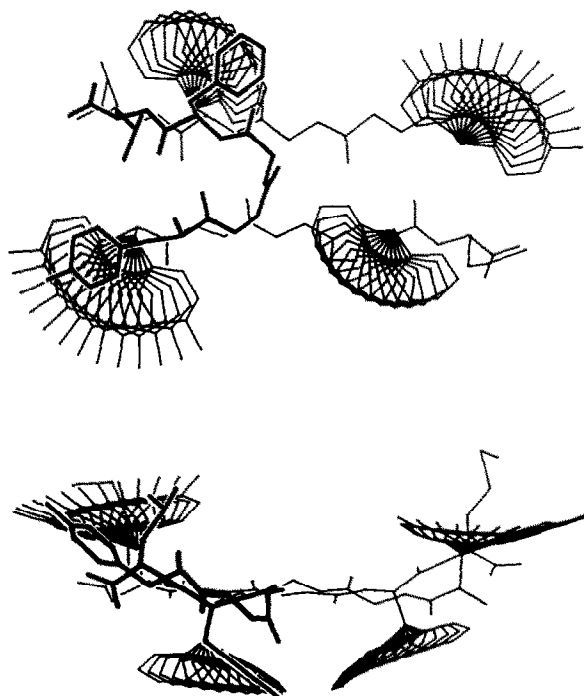


Fig.3. Conformational similarity between forms I and II. Form I was superimposed on form II. The Tyr and Phe side chains of form II were rotated at 10° intervals to fit three relative positions of Tyr, Gly and Phe to those of form I (see text).

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